Original Research Article

Effects of roasting and γ-irradiated peanuts on toxicological parameters of Wistar rats

Abstract

Effects of roasting and γ-irradiated (2, 4, 6, 8, and 10 kGy) peanuts on toxicological parameters were evaluated through feeding of Wistar rats fed for a period of eight weeks. Physical appearance, body weight, hematological, serum biochemistry and tissue pathological parameters were determined using standard methods. During the eight weeks, Wistar rats showed good physical appearance and steady weight gain, and no mortality was recorded. Haematological analysis of the Wistar rats gave no indication of anemia; packed cell volume (41.09-46.14%), haemoglobin concentration (14.01-14.80 g/L), red blood cell (11.58-12.87 μ/L), mean corpuscular volume (30.58-37.85 fl), mean corpuscular haemoglobin (11.22-12.23 pg.). Serum blood parameters including albumin, globulin, creatine and blood urea nitrogen of Wistar rats fed with rat pellet, non- and γ-irradiated peanut were not significantly different. However, total protein (4.99-5.38 g/dL), cholesterol (55.67-60.50 mg/dL), alanine amino transferase (62.56-66.56 IU/L) and aspartate amino transferase (80.50-85.50 IU/L) increased significantly ($p<0.05$) during the period of evaluation. Histopathological examination of heart, liver, lungs, intestine, and spleen revealed diffuse mild degeneration of seminiferous tubular epithelium, mild congestion of hepatic sinusoids, mild hyperplasia of goblet cells and infiltration of the lamina propria by eosinophil and lymphocytes, and sub-capsular edema. Roasted γ-irradiated peanut did not cause changes of any toxicological significance in Wistar rats.

Key words: Toxicological parameters, Roasting, Hematological parameters, Histopathological examination.

1. Introduction

Peanut (*Arachis hypogeae*) is an important legumuminous source of vegetable protein and edible oil, and annual herbaceous plant and is mainly cultivated for the seeds. Peanut has many health benefits associated with its consumption including weight gain control (Alper and

Mattes, 2002), prevention of cardiovascular diseases (Feldman, 1999), protection against Alzheimer disease (Peanut-Institute, 2002), and cancer inhibition (Awad *et al.,* 2000). Peanut contains high quality edible oil (50%), protein (25%), and carbohydrate (20%) (FAO, 2016). Peanut is grown on 27.70 million ha worldwide with total productivity of 36.45 million metric tons and an average productivity of 1.4 metric tons per ha with average yield of 12,321 hectogram per hectare in 2014 (FAOSTAT, 2018). Nigeria was third in the world production of peanut after India and China, having 8.1% of the world share (FAOSTAT, 2018) and was the largest peanut producing country in West Africa accounting for 51% of production in the region and 39% in Africa (FAOSTAT, 2018). Peanut is an excellent cash crop for both domestic market and foreign trade in several developing and developed countries (Amanullah, 2016).

Food irradiation is gaining increasing attention around the world and could be applied to commodities for the reduction of post-harvest losses of grains and legumes which vary from region to region, due to insect infestation, improper storage and preservation facilities (Falade and Kolawole 2013). Irradiation is a physical process which involves exposing food materials to ionizing radiation until a prescribed dose is absorbed, in order to improve their safety, acceptability or shelf-life (Farkas, 2011). Gamma rays (γ) are electromagnetic radiation of high energy, produced by sub-atomic particles interactions such as radioactive decay, fusion, and fission (Arvanitoyannis *et al.,* 2009). Gamma rays typically have frequencies above 10^{19}Hz, energies above 100keV, and wavelength less than 10 picometers, often smaller than an atom (Al-bachir, M. 2016). γ-irradiation was proposed as an attractive and healthy alternative process compared with chemical conventional treatments for minimizing losses and off-flavour development during storage of grains. Although Farkas and Mohacsi-Farkas, (2011) stated that the chemical structure of irradiated food is less modified than heat treated food. However, γ-irradiation might cause unfavourable changes in nutritional quality of food (Lee *et al.,* 2005; Caulfield *et al.,*2008; Chamani *et al*., 2009) and these changes depend on factors such as dose, pH, hydration state, and temperature during irradiation (Farkas and Mohacsi-Farkas, 2011). The use of γ-irradiation in the reduction of post-harvest losses was recommended up to 10kGY (FAO and WHO, 1999) and this dose have been used in reducing the viscosity of porridge from bulky starch for infant food to make available required energy and nutrient (Rombo *et al*. 2001; Lee *et al*. 2008). Considerably, Shin and Cho, (1991) reported little difference in organ weights among rat groups treated with irradiated food. In the study of Kim *et al*. (2001), he observed that the gamma-irradiated fat feeding have no influence on the plasma lipid concentrations and Roongrotchinda *et al*. (2009) established that irradiation of all of food types had no statistical implication at $p < 0.01$ on food consumption and the reproductive parameters in Sprague-Dawley rats. The absolute relationship of radiation application dose and possible change must be known in order to comprehensively assess safety and acceptability of radiation-treated peanut seeds. The objective of the study was to evaluate the effect of roasted non- and γ-irradiated (2, 4, 6, 8, and 10 kGy) peanuts on toxicological parameters of Wistar rats fed for a period of eight weeks

2. Materials and Methods

2.1 Materials and sample preparation

Seeds of four (4) peanut cultivars (Bororo white, Bororo red, Bororo hausa and Campala) were obtained from Federal College of Agriculture Minna, Nigeria. The seeds were sorted and packed in high density polyethylene Ziploc double zipper (26.8 x 27.3 cm) packages (Ziploc Brand Products, WI, USA), and were treated with γ-irradiation doses (2, 4, 6, 8, and 10 kGy, respectively) at a rate of 1.635 kGy/hr at (25 ±2^{o}C) using a ^{60}Co source, at Ghana Institute of Atomic Energy Agency, Ghana. Non irradiated (0 kGy) sample was the control and the irradiation activity of the irradiation facility was 1.44×10^{3}TBq. Subsequently, non and γ-irradiated samples were roasted at temperature 120-130^{o}C for Wistar rats feeding, and then stored at temperature of 4^{o}C.

2.2 Animal care

Forty-two healthy, weaning male Wistar rats weighing 150-170g obtained from the Central Animal House, University of Ibadan, Ibadan, Nigeria were used in this study. Prior to the commencement of the experiment, all the Wistar rats were stabilized in polypropylene cages (350×215×180 mm) with stainless steel covers, maintained at temperature 25±2^{o}C, and a 12h light/dark cycle and fed with standard rat pellet and water *ad libitum* for two weeks. This experiment was conducted according to principles stated in the Animal Care Act, and clearance was obtained from the Ethical Committee of the University of Ibadan, Ibadan, Nigeria.

2.3 Experimental designs

All Wistar rats were randomly allocated to seven experimental groups (group named after the control, non and γ-irradiation doses) each group consisting of six Wistar rats, each rat was marked with picric acid in various parts; head, tail, back, abdomen, hind limb, front limb for identification. Group 0: Wistar rats fed with non-irradiated roasted peanut (i.e. positive control group), another group was fed with only the standard rat pellet (i.e. negative control group), while Wistar rats of the other groups were fed with roasted peanuts pre-irradiated at doses (2.0, 4.0, 6.0, 8.0 and 10.0 kGy), respectively and water *ad libitum* for eight weeks. Clinical signs and physical appearance of the experimental Wistar rats were checked daily.

2.4 Body weight of Wistar rats fed on roasted and γ-irradiated peanuts

The body weight of each rat of each group was carefully measured thrice to obtain the mean, and the mean weights of the rats of each group were established to determine the standard deviation of each group. The percentage weight gain in each group of rats was recorded by dividing the change in mean body weight of those rats from week 0 to week 8 by week 0

$$\text{Weight gain (\%)} = \frac{W_2 - W_1}{W_1} \times 100$$

W_1 is mean weight of the wistar rat at week 0

W_2 is mean weight of the wistar rat at week 8

2.5 Blood glucose of Wistar rats fed on roasted and γ-irradiated peanuts

Wistar rat blood glucose determination was done in the morning using a glucose monitor with strips method. The blood was taken from the tail vein of the rats. The blood glucose was recorded at the beginning of the experiment and weekly for the period of eight weeks using ACCU-CHEK Active glucometer and its test strips (Roche Diagnostics GmbH, Germany).

2.6. Hematological analysis of Wistar rats fed on roasted and γ-irradiated peanuts

Blood samples of the rats in each group were taken weekly for hematology analysis via the abdominal aorta under ether anesthesia. The blood samples were collected into 0.2% EDTA anticoagulant bottles. The collected blood was mixed thoroughly by gentle rotation of the bottle

To enable the anticoagulant to be rapidly dissolved thereby preventing blood clotting. Blood samples were taken to the laboratory for hematology analysis. Various hematology parameters including white (WBC) and red (RBC) blood cell counts (RBC), hemoglobin concentration (Hb), mean corpuscular hemoglobin concentration (MCHC), mean corpuscular haemoglobin (MCH), platelets, mean corpuscular volume (MCV), packed cell volume (PCV), ESR and differential counts of white blood cells such as Lymphocytes, Monocytes, Neutrophils of the blood samples collected were determined using standard techniques according to Feldman *et al*. (2001). The mean of each hematological parameter of the rats of each group for the eight weeks were established.

2.7 Serum biochemical analysis of Wistar rats fed with roasted and γ-irradiated peanuts

Serum biochemistry determinations were done weekly with a Hitachi-Biochemical automatically analyzed (Model 7040E, Hitachi Co., Ltd., Tokyo, Japan). The parameter determined to include total protein (TP), blood urea nitrogen (BUN), aminotransferase (ALT), albumin (ALB), aspartate aminotransferase (AST), triglyceride (TG), creatinine (CRN), cholesterol, and globulin.

2.8 Histopathological analysis of Wistar rats fed with roasted and γ-irradiated peanuts

Animals were sacrificed under mild anesthesia with chloroform after 14-16 h overnight fast, after which some organs were harvested and examined for both gross and histological lesions. Samples of the heart, liver, kidney, intestine, lungs, testis, and spleen of Wistar rats in various groups were washed with cold saline solution (0.85% NaCl), diced and fixed in 10% phosphate-buffered formalin for 48 h, embedded in paraffin wax and sectioned at 6μm thickness following the methods of Longvah *et al*. (2000) as described by Ajayi *et al*. (2008). The tissues were stained with haematoxylin and eosin (H &E) and subsequently de-waxing for 30 min in xylene and rehydrated in descending grades of alcohols (100, 95, 80 70, 50%). The stained sections were examined under a light microscope (Nikon Corporation, Kanagawa, Japan) for changes in the tissues.

2.9 Statistical analysis

Results were expressed as the mean ± standard deviation of six separate determinations. Statistical analysis was conducted using analysis of variance (ANOVA), and means were separated using the Duncan multiple range test at the 5% level.

3. Results and discussion

3.1 Body weight measurements of Wistar rats' as affected by γ- irradiation doses

Generally, body weight of Wistar rats fed on γ-irradiated roasted peanut did not vary significantly with those fed on standard rat pellet. There were significant differences ($p<0.05$) in the percentage body weight gained in both the control and test groups (Table. 1). Mean body weight of Wistar rats fed with peanut ranged from 187.31 g (0 kGy) to 192.16 g and193.40 g (8 kGy and 10 kGy), respectively. Wistar rats fed with roasted pre-irradiated (2 kGy) peanut showed lower percentage weight gained (11.85%) than those fed with **roasted** pre-irradiated (4, 6, 8, and 10 kGy) and non-irradiated (0 kGy) peanut. The Wistar rats fed with standard rat pellet (188.35 g) had the least percentage weight gained (10.94%). A similar trend of the body weight gained by Wistar rats was reported by Falade et al. (2014) and Mohammed *et al*. (2010), in the evaluation of γ-irradiated millet, and γ-irradiated raw & processed pearl millet flour in Wistar rats, respectively. Body weight of Wistar rats fed on 10kGy γ-irradiated peanut was significantly higher than those that fed on the non-irradiated peanut. The mean body weight of the Wistar rats fed with peanut seed irradiated at 8kGy (192.16g) and 10kGy (193.40g), respectively were slightly higher than the Wistar rats fed with peanut seed irradiated at 2kGy (189.28g) and those fed with the standard rat pellet with a mean body weight of 188.35g. Magda *et al*. (2017) also confirmed body weight gained relative to the control rats during the feeding of albino rats with 5 kGy irradiated wheat grains. This showed that irradiation had no adverse effect on the nutritional adequacy of peanut on Wistar rats.

3.2 Blood glucose measurements of Wistar rats' as affected by γ- irradiation doses

The blood glucose of the Wistar rats fed with roasted non- and γ-irradiated peanut were significantly higher ($p<0.05$) than those fed with the standard rat diet. The blood glucose of Wistar rats fed with the diets increased from 112.00 mg/dL (standard rat diet) to 137.50 mg/dL (10kGy) as shown in Table 2. The blood glucose of the Wistar rat fed with 10kGy peanut was significantly higher value than others. The increase in blood glucose could be linked to weight gained in the Wistar rats as γ-irradiation had no negative effect on starch, amino acid composition and Lipid content of γ-irradiated peanuts. As reported by Rombo *et al.* (2001) and Falade and Kolawole (2011), the starch polysaccharides degraded as a result of high doses, which was capable of hydrolyzing chemical bond thereby cleaving large molecules of starch into smaller fragment of dextrin and sugars which are easily absorbed by the body, also γ –irradiation caused increase in free amino acid content, essential and non-essential amino acids of soybean when irradiated. (Kalyani *et al*., 2012) and Bhat *et al*., 2008) reported that γ-irradiation was an efficient process of maintaining the nutritional potential of seeds. The results in this study was within the normal range of blood glucose in rats as reported by Wang et al., 2011, who established 80 – 110mg/dL for fast blood glucose (FBG) and 120 – 140mg/dL for post-prandial blood glucose (PBG).

3.3 Haematological parameters measurements of Wistar rats as affected by γ-irradiation doses.

Haematology refers to the study of the numbers and morphology of the cellular elements of the blood and the use of their results in the diagnosis and monitoring of diseases (Kumar *et al*., 2011). The Mean packed cell volume (PVC) of the blood of Wistar rat after 8 weeks ranged from 41.09% to 42.42% and 46.14% in Wistar rats fed with standard rat pellet, non- irradiated, and γ-irradiated (8kGy) peanut, respectively as shown in Table 3. Mean Packed cell volume of blood of Wistar rats fed with pre-irradiated roasted peanuts at 4, 6kGy (43.55%, 43.48%) and 8kGy (46.14%) were significantly ($P<0.05$) higher than that of those fed with samples pre-irradiated at 2kGy (42.71%) and 10kGy (42.29%). Generally, packed cell volume increased significantly as the γ- irradiation doses increases. Increase in packed cell volume was observed by Purves *et al*. (2004) in Wistar rats fed with γ-irradiated samples. Also, the increased packed cell volume corresponded with increased blood volume resulting in increased red blood cell. The PCV in this study was similar to those reported for healthy Wistar rats and marine species by Ogunsanmi *et al*. (2002), indicating that the Wistar rats were not anaemic. Increased PCV of Wistar rats fed with γ-irradiated peanut seed irradiated (8kGy) indicated the normal functioning of the bone marrow (Kumar *et al*. 2011).

Packed cell volume is involved in the transportation of oxygen and absorbed nutrients, hence increased packed cell volume shows a better transportation and thus results in an increased primary and secondary polycythemia. Generally, the irradiated samples showed a high percentage of packed cell volume.

The blood haemoglobin of Wistar rats fed with peanuts ranged from 14.15 g/dl (0 kGy) to 14.80 g/dl (8kGy). Blood haemoglobin varied significantly among Wistar rats fed with standard rat diet, non-irradiated and irradiated peanut. Blood haemoglobin of Wistar rats fed with peanuts irradiated at 4, 6, and 8kGy was 14.15g/dl, 14.68g/dl, and 14.80g/dl, respectively. The blood haemoglobin content of the animals increased as the irradiation dose increased. The haemoglobin concentration of all samples was in the range of 10-15 g/dl, (recommended for haemoglobin concentration of healthy rats Ashafa *et al*., 2011; Liu et al., 2013). Haemoglobin is the iron-containing oxygen-transport metalloprotein in the red blood cells of all vertebrates and it has the physiological function of transporting oxygen to the tissues of the animals for oxidation of ingested foods so as to release energy for other body functions as well as transport carbon dioxide out of the body of animal (Liu et al., 2013). The haemoglobin concentration and haematocrit are values that show the degree of anaemia. The high values of haemoglobin concentration and haematocrit in the present study suggested adequate iron status and this could be associated to the iron content of the groundnut seeds which is a good of source of non-heme iron.

The red blood cell significantly ($p<0.05$) differed among the rats fed with standard rat pellet, non- and γ- irradiated peanut. Generally, red blood cells (RBC) of Wistar rats fed with irradiated peanut increased as irradiation dose increases. The red blood cell of Wistar rats fed on peanut ranged from 11.58 (x 10^9g/dl) (0kGy) to 12.87(x 10^9 g/dl (4 kGy). The RBC generally varied within a level comparable to the control (Wistar rats fed with standard rat pellet), though the Wistar rats fed with non-irradiated peanut had lower values whereas those fed with irradiated peanut showed a higher value. Yagi *et al.* (1998) reported that RBC of Wistar rats with higher doses of *Senna* was higher (9.91(x10^9 g/dl) than control. The red blood cells of the rats observed in this study was significantly higher than those reported by Liu *et al*. (2013) and Tasaki *et al.* (2008) which are (9.45 (x 10^9 g/dl) and (9.31(x 10^9 g/dl) respectively in their toxicity study with Wistar rats. This may be due to the conditions of the assay procedure, specifications of the animals assayed, variation in the outbred stocks and genetic drift in populations of wistar rats.

The white blood cell (WBC) of Wistar rats ranged from 13.04 x10^9/L (standard rat pellet) to 13.24x10^9/L (4 kGy) and 15.73x10^9/L (6kGy). Although the standard for WBC of rat is 6-19.0x10^9/L (Giknis and Clifford, 2008), the values obtained for the white blood cell in this study suggested that the Wistar rats had no infection. The major function of the WBC and its differential are to fight infections, defend the body by phagocytosis against invasion by foreign organisms, produce and distribute antibodies in the immune response. Also similar values of (15.10x10^9/L) and (23.30x10^9/L) were reported by Falade *et al.* (2014) and Tasaki *et al*. (2008) on haematology and serum biochemical changes induced by γ-irradiation and sub-chronic toxicity study in Wistar rats respectively. The mean white blood cell among the Wistar rats fed with non- and γ-irradiated groundnut was within the standard value. It was observed in this study that feeding of Wistar rats with roasted γ-irradiated peanuts had no significant adverse effect on their white blood cells. Generally, the mean corpuscular haemoglobin concentration (MCHC) of all the Wistar rats in the group did not differ significantly ($p<0.05$). The mean corpuscular haemoglobin concentration obtained in this study (33.0g/dL) was in agreement with the range of 32.3g/dL to 36.2g/dL (Mohammadzadeh *et al*., 2007; Tasaki *et al*., 2008; Pedrazzi*et al*., 1998) for rat but higher than 31.96g/dL reported by Sodipo *et al*. (1998) and Adam (1998).

The mean corpuscular volume (MCV) significantly differed ($p<0.05$) among the Wistar rats fed with the standard rat diet, non-irradiated and γ- irradiated peanut. The corpuscular volume of the blood of Wistar rats fed with peanuts ranged from 34.04fL (4 kGy) to 37.29fL (2 kGy) and 37.85fL (8 kGy). The mean corpuscular volume of Wistar rats fed with standard rat pellet and γ-irradiated peanuts at 6 kGy and 10 kGy were 30.58fL, 35.84fL and 36.58fL, respectively. The result obtained in this study were significantly lower than 54.25fL reported by Magda *et al*. (2017) when the authors evaluated effect of gamma irradiated wheat grains on albino rats. Higher corpuscular volume (53.50fL, 56.10Fl, 54.15fL, and 59.10 fL) was respectively reported by Tasaki *et al*. (2008); Pedrazzi *et al.* (1998); Moammadzadeh *et al.* (2007) and Liu *et al*. (2013) in their oral toxicity test using Wistar rats, this may be due to the variation in the outbred stocks and genetic drift in populations of Wistar rats. The MCV increased with increasing dose of γ-irradiation. Mean corpuscular volume is an important number on a complete blood count that can help diagnose different types of anaemia as well as other health condition. The MCV is a value that describes the average size of red blood cells (erythrocytes) in a blood or abnormalities.

The mean corpuscular haemoglobin (MCH) of Wistar rats fed with γ-irradiated 4 kGy, 8 kGy and non-irradiated (0 kG) roasted peanuts ranged from 11.22 pg to12.06 pg and 12.23 pg, respectively. The MCH was significantly affected by increased γ-irradiation dose. Magda *et al*. (2017), Liu *et al*. (2013), Mohammadzadeh *et al*. (2007), and Tasaki *et al*. (2008) have respectively reported 16.9 pg, 14.10 pg, 17.50 pg and 17.10 pg for MCH in their studies. The values in this study showed that the samples were not toxic towards the red blood cells.

Blood platelets of Wistar rats varied from 10.81 µL (standard rat diet) to 13.01 µL (10 kGy). The blood platelet of the Wistar rats was significantly affected by γ-irradiation dose. Platelet content increased in the blood of the Wistar rats fed with roasted γ-irradiated peanuts with higher doses. Increase in the platelets showed that there would be prompt clotting of blood and healing of the wound when injury was sustained. Also, An increased blood platelet helps in protein and enzyme transportation which aids blood clothing during bleeding and this protein transport hormones, minerals, vitamins, lipids and acting as buffer in regulating the acid and base concentration in blood (Jeremy, 2010).

The Erythrocyte sedimentation rate of Wistar rats was not significantly affected by γ-irradiation dose. It ranged from 4.15- 4.44 mm/h for 6 kGy and 4 kGy respectively and Wistar rats fed on standard rat pellet had the highest ESR (4.92 mm/h). The ESR is the rate of sedimentation of RBCs and is used to measure or monitoring disease activity or inflammatory disorders. The values in this study showed that γ-irradiation doses did not cause any inflammation or disease in the rat. It was documented by Wang et al., 2011 that the normal range for ESR is between 0-7 mm/h in male rats and 0-5 mm/h in female rats.

3.4 The differential leucocytes count of Wistar rats' as affected by γ- irradiation doses

The lymphocytes of Wistar rats fed with different diets ranged from 45.05% (8 kGy) to 49.96% (2 kGy). Generally, the blood lymphocytes decreased as γ- irradiation dose increased. Wistar rats fed with γ- irradiated roasted peanut at 2, 4, 6 kGy showed lymphocytes of 49.96%, 47.62%, and 47.34%, respectively. The lymphocytes in this study were lower than the 83.23% and 85.9% as reported by Liu *et al*., (2013) and Tasaki *et al.*, (2008), in multiple toxicity studies of trehalose in mice by intragastric administration and oral toxicity of ellagic acid in Wistar rats, respectively. The neutrophils content of the differential leucocytes counts significantly ($p<0.05$) varied from 49.57% (2 kGy) to 54.73% (8 kGy) among the Wistar rats fed with standard rat diet, non-and γ-irradiated peanuts. The neutrophils content increased as the irradiation doses increases. Similar trend (46.82%) was reported by Falade *et al*. (2014) on the short-term toxic effects induced by γ-irradiated millet on Wistar rats. There was no significant difference in their monocytes of the differential leucocytes count.

3.5 Serum Biochemistry Analysis of Wistar rats' as affected by γ- irradiation doses

Blood serum parameters of the rats fed with γ-irradiated peanuts may change considerably due to the importance of element (Zn2+, Na+, Mg2+, K, Fe3+ and crude protein in the peanut seeds (Alabi and Falade, 2017). Table 5 showed the effect of γ-irradiation on the serum biochemistry parameters of the Wistar rats fed on roasted non- and γ-irradiated peanuts. There were no significant differences in blood biochemistry variables such as creatine, blood urea nitrogen and albumin. Standard value of albumin for healthy rat is 1.0-2.5 g/dL (Giknis and Clifford, 2008). Albumin value of the Wistar rats fed with roasted pre-irradiated (2-10kGy) groundnuts in this study ranged from 1.0 to 2.0g/dL. Albumin is an indication of good health, it showed that there was effective regulation of the colloidal respiratory pressure of the blood, transport of fatty acid to the liver, and an indication of no damage done to the liver by feding the Wistar rats with 2-10 kGy γ-irradiated roasted peanuts. Globulin values obtained (2-4.0g/dL) for all diets were within the standard value of 1.8-4.7 g/dL (Giknis and Clifford, 2008). Low globulin resulted into dysfunction and deficiency of antibody. The remaining serum biochemistry parameters including total protein, Alanine aminotransferase (ALT), Aspartate aminotransferase (AST), triglyceride and cholesterol of the Wistar rats fed with standard rat diet, non- and γ-irradiated peanut have there mean values as; total protein (TP), ranged from 4.99 g/dL (4 kGy) to 5.31g/dL (8 kGy), 62.56 IU/L (2 kGy) to 66.56 IU/L (8 kGy), 80.50 IU/L (4 kGy) to 85.50 00IU/L (8 kGy), 72.44 mg/dL (4 kGy) to 78.50 mg/dL (8 kGy), and 55.67 mg/dl (4kGy) to 59.78 mg/dl (8 kGy). Decrease in serum biochemistry parameters were observed in Wistar rats fed with γ-irradiated peanut (2 kGy and 4 kGy) Data obtained in this study were similar to those reported by Tasaki *et al*. (2008), and Shin and Cho (1991) where serum biochemistry parameters of Wistar rats decreased as concentration of ellagic acid administered and γ-irradiation doses increased, respectively.

3.6 Histopathology

Histopathological examination revealed that there were no lesions in the kidney, heart, lungs, liver, spleen, testis, of the Wistar rats fed with both roasted non- and γ-irradiated peanut. Reversible abnormalities were infrequently observed in the intestine and testis of Wistar rats fed with both non– and γ-irradiated diet such as diffuse mild degeneration of seminiferous tubular epithelium, mild congestion of hepatic sinusoids, mild hyperplasia of goblet cells and infiltration of the lamina propria by eosinophils and lymphocytes, and subcapsular oedema in spleen. These findings were similar to those reported by Irawati and Sami, (2012) when the authors studied the effect of 45 kGy gamma irradiated foods on albino rats and found it relatively harmless on body weight gain, the toxicological impact or anatomy-pathology examination of the rats. Also, slight increase in organ weight was observed among the groups of rats fed with 5 kGy irradiated food for 8weeks by Shin and Cho, (1991).

Conclusion

The consumption of the roasted γ-irradiated peanut did not have any deleterious effect on the physical appearance, body weight gain, haematological and blood serum parameters of the test Wistar rats and this can be extrapolated to human being. Consumption of γ-irradiation treated peanut up to 10kGy did not cause changes of any toxicological significance. Irradiation treatment of peanut resulted in increased body weight and blood glucose of Wistar rats.

COMPETING INTERESTS DISCLAIMER:

Authors have declared that no competing interests exist. The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

References

Adam, S.E.I. (1998). Toxic effects of *Francoeuriacrispa* in Wistar rats. Phytotherapy Research, 12, 476-479.

Ajayi, I.A., Oderinde, R.A., Taiwo, V.O., Agbedana, E.O. (2008). Short term toxicological Evaluation of *Terminalia catappa, Pentaclethramacrophylla and Calophylluminophyllum* seed oils in Wistar rats. Journal of Food Chemistry, 106, 458-465

Alabi, A.O and Falade, K.O. (2017). Physical, Proximate and Functional Properties of Flour and Protein Isolate from Four γ-irradiated groundnut Cultivars. *Journal of Food Processing & Technology 8:12*

Al-bachir, M. (2016). Evaluation of the effect of gamma irradiation on microbial, chemical and Sensorial properties of peanut (*Arachis hypogaea* L.) seeds. *Acta Science pol. Technology Aliment.*, 15(2): 171-179. DOL: 10.17306/J.AFS.2016.2.17.

Alper, C.M.,and Matters, R.D. (2002). Effects of chronic peanut consumption on energy balance and Hedonics. International Journal of Obesity, 26: 1129-1137.

Amanullah. "International Year of Pulses (2016)". *EC Agriculture Editorial column, 01 (2016)*:05-07.

Arvanitoyannis, I.S., Stratakos, A., and Tsarouhas, P. (2009). Irradiation applications in vegetables and fruits: a review, Critical Review in Food Science and Nutrition, 49,427-462.

Ashafa, A.O.T, Sunmonu, T.O, Afolayan, A.J (2011) Effects of leaf and berryextracts of Phytolaccadioica L. on haematological and weightparameters of WistarWistar rats. Afr J Pharm Pharmacol 5(2):150–154

Awad, A. B., Chan, K.C., and Fink, C.S. (2000). Peanuts as a source of B-sitosterol, a sterol with anticancer properties. Journal of Nutrition and Cancer, 36: 238-241.

Bhat, R, Sridhar, K.R., Young, C., Bhagwath, A. A., Ganesh, S. (2008), Composition and functional properties of raw and electron beam-irradiated Mucunapruriens seed. Int J. Food sci Technology 43: 1338-1351.

Caulfield CD, Cassidy JP, Kelly JP (2008) Effects of gamma irradiationand pasteurization on the nutritive composition of commerciallyavailable animal diets. J Am Assoc Lab AnimSci 47:61–66.

Chamani M, Molaei M, Foroudy F, Janmohammadi H, Raisali G (2009). The effect of autoclave processing and gamma irradiation on apparentileal digestibility in broiler breeders of amino acids from canolameal. Afr J Agric Res 4:592–598.

Falade, K.O.and Kolawole, T.A. (2011). Physical, Functional, and Pasting properties of different Maize (Zea mays) cultivars as modified by an increase in ɤ-irradiation doses. International journal of Food Science and Technology, 47, 801-807.

Falade K.O, Kolawole T.A. (2013a) Effect of γ-irradiation on colour,functional and physicochemical properties of pearl millet[Pennisetumglaucum (L) R. Br.] cultivars. Food BioprocessTechnol 6:2429–2438. doi:10.1007/s11947-012-0981-8.

Falade, K.O.,Oweghoro , B.O., Lasisi, T.O., Emikpe, B.O. (2014), Haematological, Serum biochemical and tissue pathological changes induced by γ-irradiation millet. Comp. ClinPathol Springer-Verlag London.

Farkas, J. (2006). Irradiation for better foods. Trends in Food Science and Technology, 17: 148-152.

Farkas, J., and Mohacsi-Farkas, C. (2011). History and future of food irradiation. *Trends in Food Science & Technology*, 22: 121-126.

FAO⁄IAEA⁄WHO (1999) High-dose irradiation: wholesomeness of foodirradiated with doses above 10 kGy. Report of a joint FAO ⁄ IAEA ⁄WHOstudy group. Food andAgriculture Organisation of the UnitedNations, Rome.

FAO. (2016) “International Year of Pulses 2016”. FAO.Org/pulse- 2016. 15384E/ 1/02/16.

FAOSTAT, (2018). Food and Agriculture Organization Statistical data. Retrieved April 20th 2020, from http://faostat.fao.org/.

Feldman, F.K., Zinkf, J.G. and Jain, N.C. (2000). Schalm’s Veterinary Hematology, 5th ed., Lippincott Williams & Wilkins. Philadelphia, PA.,

Feldman, E.B. (1999). Assorted monounsaturated fatty acids promote healthy hearts. American Journal of Clinical Nutrition, 70:953-95

Giknis, M.L.A., and Clifford, C.B. (2008). Clinical laboratory parameters for Crl: CD (SD) Rats, *Charles River Laboratories*.

Irawati, Z., Sani, Y. (2012). Feeding studies of radiation sterilization ready to eat foods on Sprague Dawley rats in vivo, *Natural Science*, 4(2), 116–122.

Jeremy, E.K. (2010). Cardiovascularefficiency Vs Nutritional Deficiency.International foundation for health and nutrition in San Diego. Journal of Veterinary Obstetic 858: 2488-2533.

Kalyani, B., Manjula, K. D. Kusuma, L. (2012). Food consumption pattern and weight gain of albino rats fed with irradiated and non-irradiated diet, Indian J.L. Sci., 2012, 2(1), 73–75.

Kim, E., Jeon, S.M., and Choi, M.S. (2001), Effects of γ-irradiated fats on plasma lipid concentrations and hepatic cholesterol metabolism in rats, Annals of Nutrition and Metabolism, 45(4), 152– 158.

Kumar, K.U., Sharief, S.D., Rajkumar, R.,Ilango,B. and Sukumar, E.(2011).Influence of Lantana aculeate stem extract on Haematological parameters in Wistar rats. Advances in Bioresearch, 2 (1) 79-81.

Lee, S., Lee, M., Song, K. (2005). Effect of gamma-irradiation on thephysicochemical properties of gluten films. Food Chem 92:621–925.

Lee J, Kim J, Oh S, Byun E, Yook H, Kim M, Kim K, Byun M (2008) Effect of gamma irradiation on viscosity reduction ofcereal porridges for improving energy density. RadiatPhys.Chem 77:352–356.

Liu, M., Zhoug, M., Ye, H., Lin, S., Yang, Y., Wang, L., Jones, G. and Trang, H (2013). Multiple toxicity studies of trehalose in Mice by Intragastric administration. Journal of Food Chemistry, 136, 485-490.

Longvah, T., D'eosthale, Y.G., and Uday Kumar, P. (2000). Nutritional and short term toxicological evaluation of *Perilla* seed oil. Food Chemistry, 70, 13-16.

Magda, S., Hanafy, N. and Alywa, M.A. (2017). Effect of feeding Albino rats with irradiated wheat grains on body weight and some haematological parameters. *Romanian Journal of Biophysics* Vol 26 (3) 175-184.

Mohammadzadeh, S. Mohammed, S., Manoo, C.H., Ahmad-khamha. R., Samadi, N., Ostad, S.N., (2007). Chemical composition, Oral toxicology and antimicrobial activity of Iranian Polis. *Journal Food Chemistry*, 103, 1097-1103.

Mohamed EA, Ahmed IAM, Yagoub AEA, Babiker EE (2010) Effectsof radiation process on total protein and amino acids composition ofraw and processed pearl millet flour during storage. Int J Food SciTechnol 45:906–912.

Ogunsanmi, A.0., Ozegbe, P.C., Ogunjobi, O., Taiwo, V.O. and Adu, J.O. (2002). Haematology plasms Biochemistry and whole blood minerals of the Captive adult grasscutter (*ThryonomysswinderiamusTemminck).* Tropical Veterinary,20.27-35.

Peanut Institute. 2011. Types of Peanut oil. Available from http://www.*peanutinstitute.*

org/health-and-nutrition. / Accessed October 20, 2011.

Pedrazzi, H.P.A., Rodrigues, E.R., Bastos, J.K. (1998). Acute preclinical Toxicity study of *Zanthoxylumnaranjillo* extract. Phytotherapy Research, 12, 512-516.

Purves W. K, Sadava D, Orians G. H, Heller, H. C (2004) Life. The Science of Biology (7th ed.). Mass: Sinauer Associates, Sunderland, p 954.

Roongrotchinda, K, Kengkoom, K, and Ampawong, S. (2009). Effect of pasteurized, irradiated, and autoclaved food on reproductive performance and growth rate in Sprague-Dawley rat, In: Proceedings of the 47th Kasetsart University Annual Conference, Kasetsart, pp. 261–268

Rombo, G.O, Taylor J. R. N, Minnaar, A (2001). Effect of irradiation, with andwithout cooking of maize and kidney bean flours, on porridgeviscosity and in vitro starch digestibility. J Sci Food Agric 81:497–502.

Shin, K.S. and Cho, J.H, (1991). Influence of irradiated diets on the growth and blood chemical values of rats, *Korean Journal of Veterinary Public Health*, 15(1), 143–154.

Sodipo, O.A., Effraim,K.D. and Emmagun, E. (1998). Effect of aqueous leaf extracts *Cassia alata*. (*Linn*) on some Haematological indices in Albino Wistar rats. Phytotherapy Research, 12, 431-433.

Tasaki, M.,Umemura, T., Maeda, M., Ishii,Y., Okamura, T. Inoue, T, Kuroiwa, Y., Hirose, M., Nishikawa (2008). Safety assessmentof ellagic acid, a food additive, in a subchronic toxicity study using F344 Wistar rats. Journal of Food and Chemistry Toxicology, 46: 119-1124.

Wang, Z., Yang, Y., Xiang, X., Zhu, Y., Men, J and He, M. (2010). Estimation of the normal range of blood glucose in rats. Journal of hygiene research, 39 (2) 133-142.

Yagi, S.M., Tigani, S. E and Adam, S.E.I. (1998). Toxicity of *sennaobtusifolia* fresh and fermented leaves (kawal*)*, *Senna alata leaves* and some products from *sennaalata* on Wistar rats. Phytotherapy Research, 12, 324-350.

Table 1: Body weight (g) of wistar rats' as affected by γ- irradiation doses.

Week		Irradiated peanut diet (kGY)					
	Standard diet	0	2	4	6	8	10
0	169.77±1.85^{a}	166.23±2.05^{a}	169.22±1.37^{a}	167.78±1.26^{a}	165.88±1.27^{a}	168.48±1.92^{a}	168.72±2.93^{a}

1	170.23±2.12^{a}	169.88±2.07^{a}	170.07±1.27^{a}	171.22±2.44^{a}	169.65±1.28^{a}	170.23±1.24^{a}	171.82±2.62^{a}
2	172.88±1.96^{ab}	171.27±2.69^{b}	172.28±1.69^{ab}	173.63±1.98^{ab}	172.63±1.7^{ab}	173.21±1.28^{ab}	174.35±2.87^{a}
3	175.52±2.07^{ab}	173.22±3.66^{c}	174.23±2.05^{b}	176.28±1.63^{a}	176.82±2.15^{a}	177.23±1.15^{a}	177.81±2.76^{a}
4	179.25±2.25^{b}	176.07±1.42^{c}	177.57±3.05^{bc}	178.22±1.05^{b}	179.65±2.39^{b}	179.18±1.46^{b}	181.33±2.71^{a}
5	181.62±2.24^{bc}	179.61±1.34^{c}	180.45±2.24^{c}	180.05±1.44^{c}	183.25±1.58^{b}	182.55±2.76^{b}	185.72±2.57^{a}
6	183.23±2.37^{bc}	180.82±2.28^{d}	182.23±2.58^{c}	182.38±2.18^{c}	185.63±3.11^{b}	185.72±1.87^{b}	188.65±3.35^{a}
7	185.03±2.96^{c}	183.40±2.59^{d}	185.45±3.67^{c}	185.65±1.97^{c}	188.23±2.21^{b}	188.36±2.72^{b}	190.66±3.41^{a}
8	188.35±2.17^{c}	187.31±1.96^{c}	189.28±1.05^{b}	189.51±1.85^{b}	190.65±2.33^{b}	192.16±2.82^{b}	193.40±4.18^{a}
Change in weight	10.94%	12.68%	11.85%	12.95%	14.93%	14.39%	14.63%

* Mean ± S.D (n=6-number of rat fed for 8 weeks) across a row with different superscript are significantly different with a>b>c>d. Mean separation done with Duncan multiple range test

Table 2: Blood glucose (mg/dL) measurements of wistar rats' as affected by γ- irradiation doses

Week		Irradiated peanut diet (kGY)					
	Standard diet	0	2	4	6	8	10
0	119.17±2.54^{a}	120.50±3.21^{a}	125.83±2.22^{a}	122.17±2.04^{a}	119.17±1.69^{a}	120.17±3.89^{a}	126.50±0.15^{a}
1	112.83±2.67^{bc}	123.00±1.18^{a}	123.00±1.06^{a}	119.17±1.46^{b}	125.50±0.55^{a}	116.67±2.64^{b}	123.17±1.34^{a}
2	117.17±1.65^{b}	125.50±1.88^{a}	127.67±2.18^{a}	126.17±1.74^{a}	124.33±2.98^{a}	116.50±1.57^{b}	125.67±1.19^{a}
3	119.00±1.53^{bc}	131.83±1.61^{a}	130.00±1.77^{a}	129.67±1.66^{ab}	126.30±2.27^{ab}	124.17±1.60^{b}	133.30±0.15^{a}
4	115.00±1.14^{c}	131.30±2.02^{ab}	137.00±1.21^{a}	129.50±1.44^{b}	130.51±2.03^{ab}	121.17±1.60^{bc}	133.40±1.37^{ab}
5	110.83±2.41^{c}	128.00±3.51^{b}	131.50±3.63^{ab}	127.35±2.95^{b}	137.83±1.45^{a}	125.00±2.27^{b}	133.70±1.05^{ab}

6	108.17 ± 2.99^{d}	132.20 ± 1.74^{ab}	126.01 ± 1.12^{bc}	129.50 ± 1.36^{b}	132.80 ± 1.76^{ab}	122.80 ± 1.95^{c}	136.00 ± 1.19^{a}
7	115.58 ± 3.13^{b}	125.60 ± 1.43^{ab}	124.67 ± 2.39^{b}	130.67 ± 2.09^{a}	133.00 ± 1.47^{a}	123.00 ± 1.75^{b}	134.60±0..31^{a}
8	112.00 ± 2.27^{c}	114.00 ± 3.66^{c}	124.21 ± 1.52^{b}	132.00 ± 1.02^{ab}	122.00 ± 2.93^{b}	125.80 ± 1.98^{b}	137.50 ± 0.58^{a}

* Mean ± S.D (n=6-number of rat fed for 8 weeks) across a row with different superscript are significantly different with a>b>c>d. Mean separation done with Duncan multiple range test

Table 3. Haematological parameters measurements of Wistar rats as affected by γ-irradiation doses

		Irradiated Groundnut diet (kGy)					
Blood index	Standard pellet	0	2	4	6	8	10
Haemoglobin (g/L)	14.11 ± 1.22^{a}	14.15 ± 1.49^{a}	$14.15+1.48^{a}$	$14.15+0.91^{a}$	$14.68+1.01^{a}$	$14.80+1.15^{a}$	$14.01+1.39^{a}$
PCV (%)	41.09 ± 3.33^{c}	42.42 ± 4.01^{b}	42.72 ± 4.48^{b}	43.55 ± 2.69^{b}	43.48 ± 3.09^{b}	46.14 ± 3.53^{a}	42.29 ± 3.87^{b}
RBC ($x10^{12}$ g/L)	11.75 ± 1.31^{b}	$11.58+1.26^{b}$	$11.82+1.83^{b}$	$12.87+1.36^{a}$	$12.31+1.77^{a}$	$12.62+1.71^{a}$	$11.79+1.65^{b}$
MCV(fl)	30.58 ± 4.12^{c}	36.67 ± 5.90^{a}	37.29 ± 4.00^{a}	34.04 ± 3.83^{b}	35.84 ± 4.71^{b}	37.85 ± 4.98^{a}	36.58 ± 3.89^{a}
MCH (pg)	11.56 ± 1.61^{b}	12.23 ± 1.92^{a}	11.30 ± 2.07^{b}	11.22 ± 1.55^{b}	11.65 ± 1.61^{ab}	12.06 ± 1.51^{a}	11.60 ± 1.79^{ab}
MCHC (%)	32.64 ± 0.01^{a}	33.01 ± 0.00^{a}	32.73 ± 0.60^{a}	32.82 ± 0.10^{a}	32.78 ± 0.30^{a}	33.01 ± 0.01^{a}	33.02 ± 0.10^{a}
WBC($x10^{9}$/L)	13.04 ± 2.97^{c}	15.07 ± 3.97^{a}	15.02 ± 3.58^{a}	13.24 ± 3.06^{c}	15.73 ± 4.57^{a}	14.55 ± 3.38^{b}	13.54 ± 3.93^{c}
Platelets(μ/L)	10.81 ± 1.15^{b}	10.91 ± 0.89^{b}	10.85 ± 1.10^{b}	11.33 ± 0.93^{ab}	11.68 ± 1.01^{a}	12.02 ± 1.33^{a}	13.01 ± 1.47^{a}
ESR (mm/h	4.92 ± 0.95^{a}	4.43 ± 1.03^{a}	4.21 ± 1.12^{a}	4.44 ± 1.62^{a}	4.15 ± 1.07^{a}	4.34 ± 1.26^{a}	4.26 ± 1.54^{a}

Note: * Mean ± S.D (n=6-number of rat fed for 8 weeks) across a row with different superscript are significantly different with a>b>c>d. Mean separation done with Duncan multiple range test.

Table 4: Mean Differential Leucocytes count of Wistar rats' as affected by γ-irradiation doses

		Irradiated groundnut diets (kGy)					
Blood Index	Rat pellet	0	2	4	6	8	10
Lymphocytes (%)	46.78±0.97^{b}	45.13±2.82^{c}	49.76±1.25 a	47.62±1.05 b	47.34±1.55 b	44.55±1.08 c	45.54±1.40 c
Monocytes (%)	1.10±0.02^{a}	1.09±0.01^{a}	1.07±0.02a	0.92±0.01	1.11 ±0.02a	1.02 ±0.01a	0.91 ±0.03a
Neutrophils (%)	52.96±0.92^{b}	53.68±2.88^{a}	49.57±1.24 c	52.11±0.94 b	51.62±2.54 b	54.73±1.21 a	54.15±1.46 a

Note: * Mean ± S.D (n=6-number of rat fed for 8 weeks) across a row with different superscript are significantly different with a>b>c>d. Mean separation done with Duncan multiple range test.

Table 5: Serum Biochemistry Analysis of Wistar rats' as affected by γ- irradiation doses

Serum Indices	**Rat pellet**	**0**	**2**	**4**	**6**	**8**	**10**
Total Protein (g/dL)	5.26±0.90^{a}	5.38±0.81^{a}	5.05±0.91^{a}	4.99±1.10^{a}	5.25±0.82^{a}	5.31±0.85^{a}	5.17±0.95^{a}
Albumin (g/dL)	1.56±0.51^{a}	1.67±0.46^{a}	1.56±0.51^{a}	1.56±0.51^{a}	1.50±0.51^{a}	1.56±0.51^{a}	1.56±0.51^{a}
lobulin (g/dL)	3.44±0.71^{a}	3.56±0.71^{a}	3.17±0.62^{a}	3.22±0.73^{a}	3.67±0.77^{a}	3.61±0.70^{a}	3.44±0.71^{a}
Cholesterol (mg/dL)	60.50±9.72^{a}	61.28±12.55^{a}	56.22±13.10^{a}	55.67±14.63^{a}	59.56±11.82^{a}	59.78±12.76^{a}	58.50±15.08^{a}
Triglyceride (mg/dL)	77.56±13.26^{a}	79.50±13.0^{a}	74.22±16.21^{a}	72.44±16.98^{a}	77.78±13.26^{a}	78.50±14.18^{a}	76.56±16.21^{a}

Creatine (mg/dL)	1.44±0.51[a]	1.56±0.51[a]	1.44±0.51[a]	1.44±0.51[a]	1.56±0.51[a]	1.56±0.51[a]	1.56±0.51[a]
ALT (IU/L)	65.17±15.15[a]	67.67±13.42[a]	62.56±15.50[a]	62.89±17.12[a]	65.50±14.27[a]	66.56±14.22[a]	65.89±14.98[a]
AST (IU/L)	82.22±19.25[a]	86.72±14.86[a]	81.11±18.44[a]	80.50±20.76[a]	83.72±17.04[a]	85.50±16.21[a]	83.89±17.83[a]
BUN (mg/dL)	1.22±0.43[a]	1.33±0.49[a]	1.28±0.46[a]	1.33±0.48[a]	1.44±0.51[a]	1.44±0.51[a]	1.44±0.51[a]

Note: * Mean ± S.D (n=6-number of rat fed for 8 weeks) across a row with different superscript are significantly different with a>b>c>d. Mean separation done with Duncan multiple range test. ALT; Alanine aminotransferase ,AST; Aspartate aminotransferase, BUN; Blood Urea nitrogen